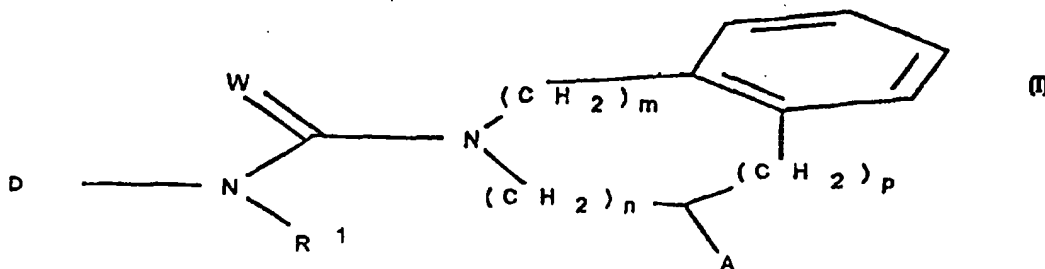




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(21) International Application Number: PCT/DK96/00059 (22) International Filing Date: 6 February 1996 (06.02.96) (30) Priority Data: 0147/95 9 February 1995 (09.02.95) DK (71) Applicant (for all designated States except US): NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bagsvaerd (DK). (72) Inventor; and (75) Inventor/Applicant (for US only): ANKERSEN, Michael [DK/DK]; Dalgas Have 34, 1. th., DK-2000 Frederiksberg (DK). (74) Common Representative: NOVO NORDISK A/S; Corporate Patents, Novo Allé, DK-2880 Bagsvaerd (DK).		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AZ, BY, KG, KZ, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>

(54) Title: COMPOUNDS WITH GROWTH HORMONE RELEASING PROPERTIES

**(57) Abstract**

There are disclosed novel compounds of general formula (I) which compounds of formula (I) promote the release of growth hormone in humans and animals. This property can be utilized to promote the growth of food animals to render the production of edible meat products more efficient, and in humans, to increase the status of those afflicted with a lack of a normal secretion of natural growth hormone. Growth promoting compositions containing such compounds of formula (I) as the active ingredient thereof, methods of stimulating the release of growth hormone as well as use of such compounds of formula (I) are also disclosed.

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COMPOUNDS WITH GROWTH HORMONE RELEASING PROPERTIES

FIELD OF INVENTION

The present invention relates to novel compounds, compositions containing them, and their use for treating medical disorders 5 resulting from a deficiency in growth hormone.

BACKGROUND OF THE INVENTION

Growth hormone is a hormone which stimulates growth of all tissues capable of growing. In addition, growth hormone is known to have a number of effects on metabolic processes, e.g., 10 stimulation of protein synthesis and free fatty acid mobilization and to cause a switch in energy metabolism from carbohydrate to fatty acid metabolism. Deficiency in growth hormone can result in a number of severe medical disorders, e.g., dwarfism.

15 Growth hormone is released from the pituitary. The release is under tight control of a number of hormones and neurotransmitters either directly or indirectly. Growth hormone release can be stimulated by growth hormone releasing hormone (GHRH) and inhibited by somatostatin. In both cases the 20 hormones are released from the hypothalamus but their action is mediated primarily via specific receptors located in the pituitary. Other compounds which stimulate the release of growth hormone from the pituitary have also been described. For example arginine, L-3,4-dihydroxyphenylalanine (L-Dopa), 25 glucagon, vasopressin, PACAP (pituitary adenylyl cyclase activating peptide), muscarinic receptor agonists and a synthetic hexapeptide, GHRP (growth hormone releasing peptide) release endogenous growth hormone either by a direct effect on the pituitary or by affecting the release of GHRH and/or

somatostatin from the hypothalamus.

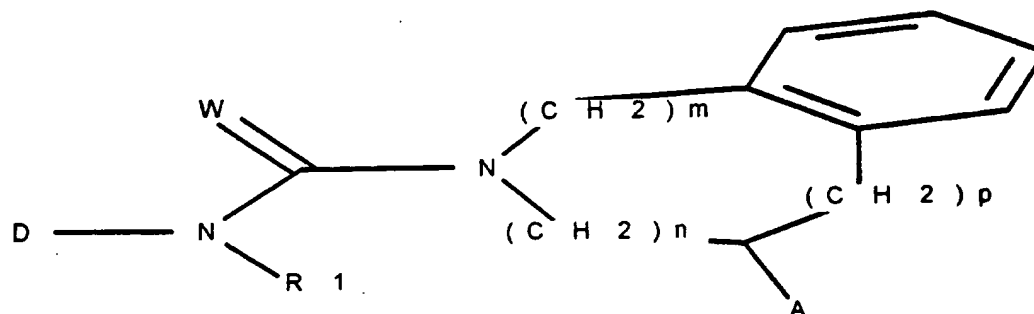
In disorders or conditions where increased levels of growth hormone is desired, the protein nature of growth hormone makes anything but parenteral administration non-viable. Furthermore, 5 other directly acting natural secretagogues, e.g., GHRH and PACAP, are longer polypeptides for which reason oral administration of them is not viable.

The use of certain compounds for increasing the levels of growth hormone in mammals has previously been proposed, e.g. in 10 EP 18 072, EP 83 864, WO 89/07110, WO 89/01711, WO 89/10933, WO 88/9780, WO 83/02272, WO 91/18016, WO 92/01711, WO 93/04081 WO 95/17422, WO 95/17423 and WO 95/14666.

The composition of growth hormone releasing compounds is important for their growth hormone releasing potency as well as 15 their bioavailability. It is therefore the object of the present invention to provide compounds with growth hormone releasing properties which have improved properties relative to known peptides of this type.

SUMMARY OF THE INVENTION

20 Accordingly, the present invention relates to a compound of general formula I



wherein

m, n and p are independently 0, 1 or 2,

m + n + p is 2,

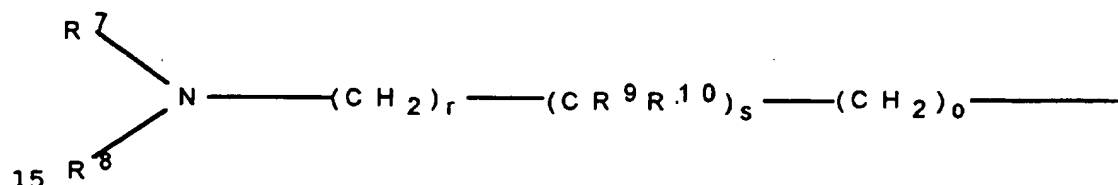
W is =S, =O, =NH or =N(CN),

5 R¹ is hydrogen, aryl or C₁₋₆-alkyl optionally substituted with halogen, amino, hydroxy or aryl;

A is aryl or C₁₋₆-alkyl optionally substituted with aryl, -CONR³R⁴ or

-CONR³CHR⁴CONR⁵R⁶, wherein R³, R⁴, R⁵, and R⁶ independently are
 10 hydrogen, aryl or C₁₋₆-alkyl optionally substituted with halogen, amino, hydroxy or aryl,
 with the proviso that at least one of R³, R⁴, R⁵ or R⁶ is an aryl or C₁₋₆-alkyl substituted with aryl;

D is



wherein R⁷, R⁸, R⁹ and R¹⁰ independently are hydrogen or C₁₋₆-alkyl optionally substituted with halogen, amino, hydroxy or aryl, R⁷ and R⁸, R⁹ and R¹⁰, R⁷ and R⁹ or R⁸ and R¹⁰ optionally forming -(CH₂)_i-U-(CH₂)_j-, wherein i and j are independently 1,
 20 2 or 3,

U is -O-, -S- or a valence bond,

o and r are independently 0, 1, 2, 3 or 4,

s is 0 or 1,

r + s + o is 2, 3 or 4;

25 or a pharmaceutically acceptable salt thereof.

It is believed that compounds of formula I exhibit an improved bioavailability because they contain no amide bonds susceptible to cleavage by proteolytic enzymes. The increased resistance to proteolytic degradation combined with the reduced size of the compounds of the invention in comparison with known growth hormone releasing compounds is expected to improve their bioavailability compared to that of the compounds suggested in the prior literature.

In the above structural formulas and throughout the present specification, the following terms have the indicated meanings:

The C_{1-6} -alkyl groups specified above are intended to include those alkyl groups of the designated length in either a linear or branched or cyclic configuration. Examples of linear alkyl are methyl, ethyl, propyl, butyl, pentyl, and hexyl. Examples of branched alkyl are isopropyl, sec-butyl, tert-butyl, isopentyl, and isohexyl. Examples of cyclic alkyl are cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

Especially preferred C_{1-6} -alkyl groups are the C_{1-3} -alkyl groups. Preferred C_{1-3} -alkyl groups are methyl, ethyl, isopropyl, and cyclopropyl.

The C_{1-6} -alkoxy groups specified above are intended to include those alkoxy groups of the designated length in either a linear or branched or cyclic configuration. Examples of linear alkoxy are methoxy, ethoxy, propoxy, butoxy, pentoxy, and hexoxy. Examples of branched alkoxy are isopropoxy, sec-butoxy, tert-butoxy, isopentoxy, and isohexoxy. Examples of cyclic alkoxy are cyclopropyloxy, cyclobutyloxy, cyclopentyloxy and cyclohexyloxy.

Especially preferred C_{1-6} -alkoxy groups are the C_{1-3} -alkoxy

groups. Preferred C₁₋₃-alkoxy groups are methoxy, ethoxy, isopropoxy, and cyclopropyloxy.

The C₁₋₆-alkylamino groups specified above are intended to include those alkylamino groups of the designated length in either a linear or branched or cyclic configuration. Examples of linear alkylamino are methylamino, ethylamino, propylamino, butylamino, pentylamino, and hexylamino. Examples of branched alkylamino are isopropylamino, sec-butylamino, tert-butylamino, isopentylamino, and isohexylamino. Examples of cyclic alkylamino are cyclopropylamino, cyclobutylamino, cyclopentylamino and cyclohexylamino.

Especially preferred C₁₋₆-alkylamino groups are the C₁₋₃-alkylamino groups. Preferred C₁₋₃-alkylamino groups are methylamino, ethylamino, isopropylamino, and cyclopropylamino.

In the present context, the term "aryl" is intended to include aromatic rings, such as carbocyclic and heterocyclic aromatic rings selected from the group consisting of phenyl, naphthyl, pyridyl, 1-H-tetrazol-5-yl, thiazolyl, imidazolyl, indolyl, pyrimidinyl, thiadiazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiophenyl, quinolinyl, pyrazinyl, or isothiazolyl, optionally substituted by one or more C₁₋₆-alkyl, C₁₋₆-alkoxy, halogen, amino or aryl. Aryl is preferably phenyl, thienyl, imidazolyl, pyridyl, indolyl, quinoline or naphthyl optionally substituted with halogen, carboxamido, tetrazolyl, oxadiazolyl, thiadiazolyl, amino, hydroxy, C₁₋₆-alkyl or C₁₋₆-alkoxy. The term "halogen" is intended to include Cl, F, Br and I.

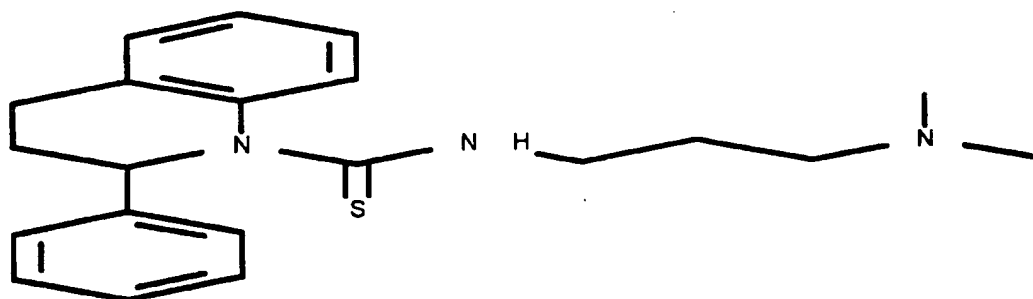
The compounds of the present invention may have one or more asymmetric centres and stereoisomers in the form of separated, pure or partially purified stereoisomers or racemic mixtures thereof are intended to be included in the scope of the

invention.

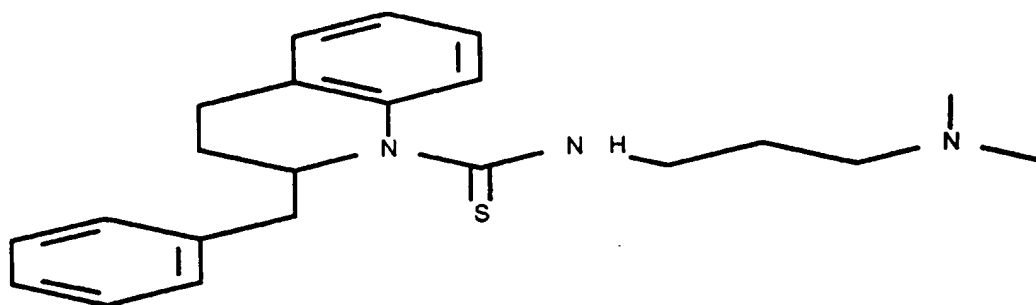
DETAILED DESCRIPTION OF THE INVENTION

Examples of specific compounds of the present invention are

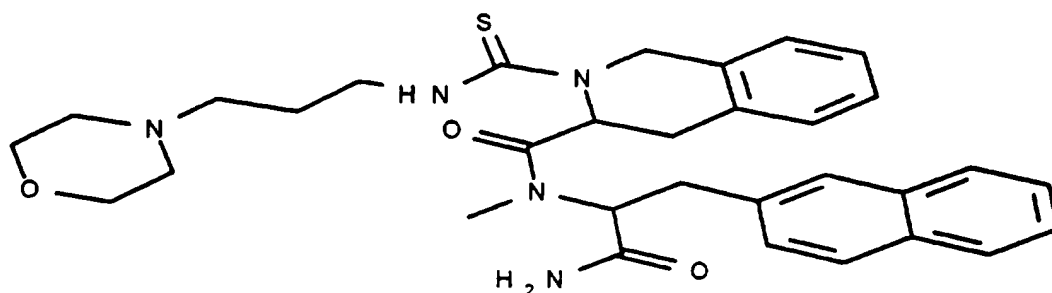
2-Phenyl-3,4-dihydro-2H-quinoline-1-carbothioic acid (3-
5 (dimethylamino)propyl) amide



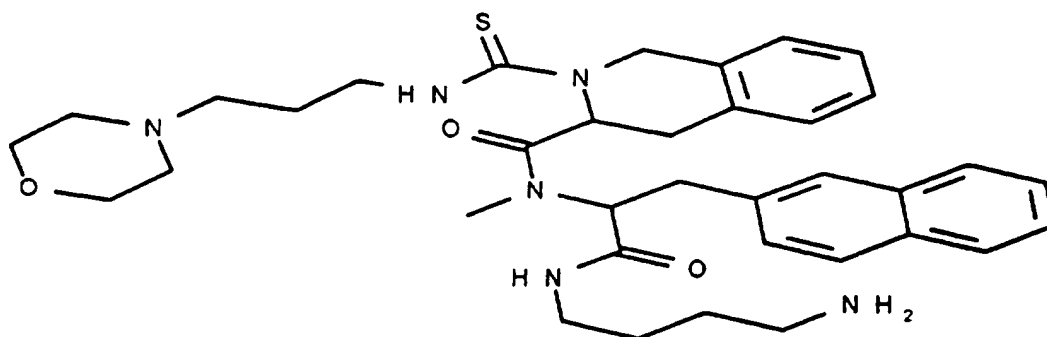
Benzyl-3,4-dihydro-2H-quinoline-1-carbothioic acid (3-
(dimethylamino)propyl) amide



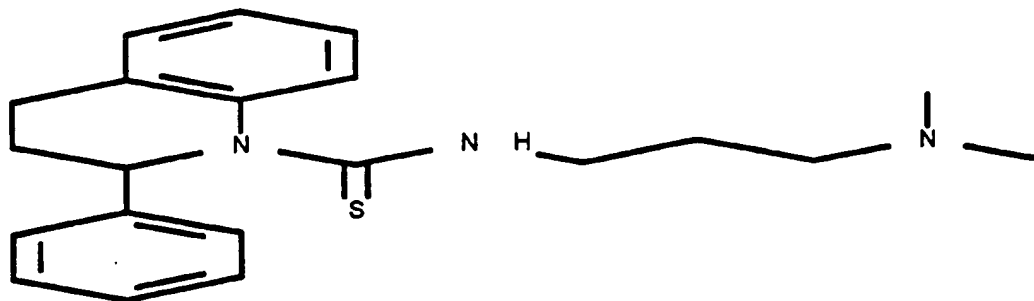
1-(3-((Morpholin-4-yl)propyl)thiocarbamoyl)-1,2,3,4-tetrahydro-1H-quinoline-2-carboxylic acid N-(1-carbamoyl-2-(naphth-1-yl)ethyl)-N-methylamide



5 2-(3-((Morpholin-4-yl)propyl)thiocarbamoyl)-1,2,3,4-tetrahydro-1H-isoquinoline-3-carboxylic acid N-[1-((4-aminobutyl) carbamoyl)-2-(naphth-1-yl)ethyl]-N-methylamide



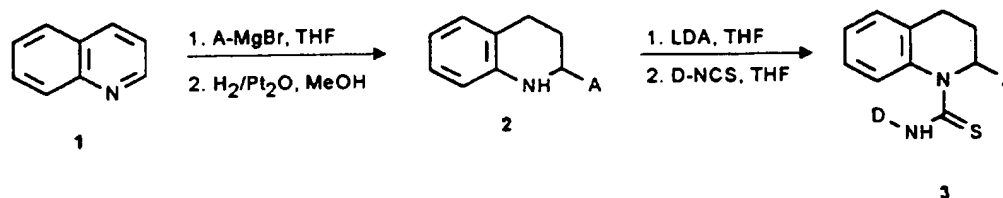
2-Phenyl-3,4-dihydro-2H-quinoline-1-carbothioic acid (3-(morpholin-4-yl)propyl) amid



5 Compounds of formula I may be prepared from natural or non-natural amino acid residues as shown in one of the following reaction schemes. The non-natural amino acid residues may be prepared according to methods known to those skilled in the art.

10 General Method A

Reaction Scheme I:

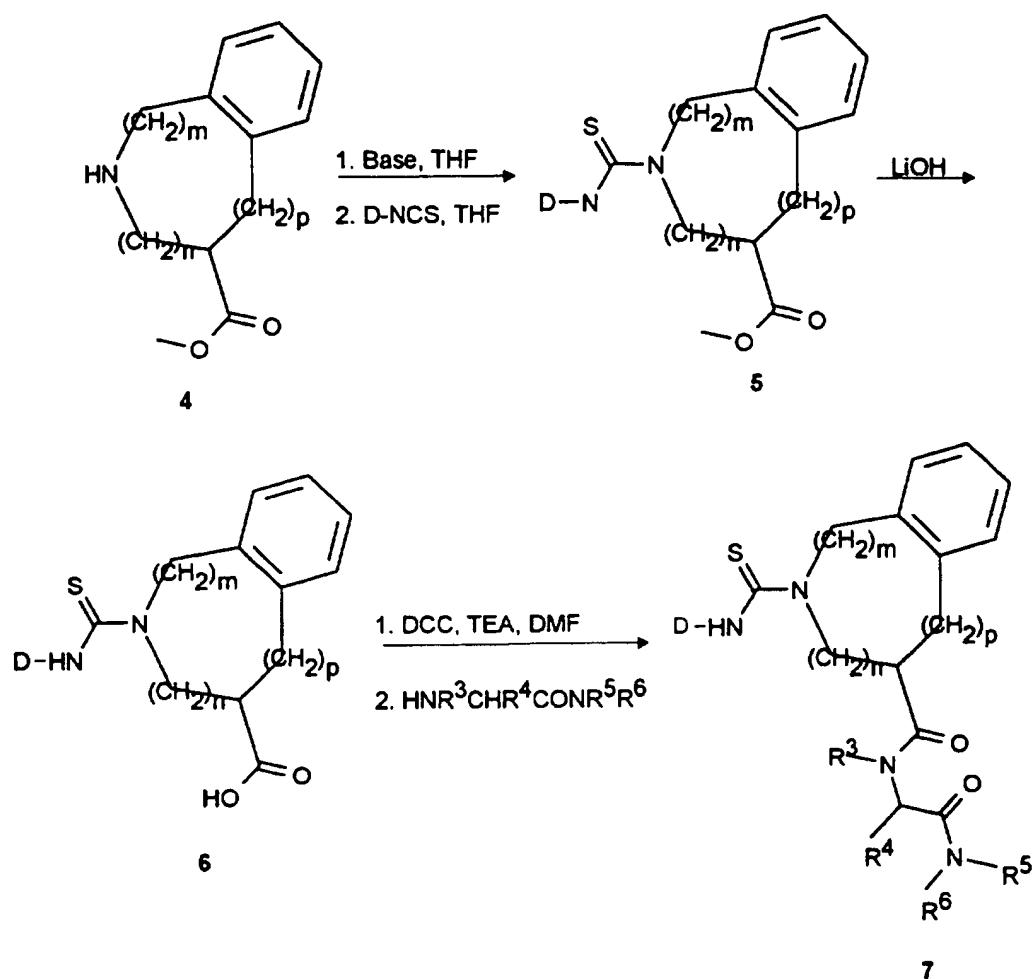


Compounds of formula I may be prepared as shown in reaction scheme I starting with quinoline 1 and a Grignard reagent such as arylmagnesium bromide, which may be prepared from the

corresponding bromide after methods known for those skilled in the art, or an organolithium compound such as phenyllithium in an appropriate solvent such as THF. The addition product may then be reduced by a reducing agent such as hydrogen over 5 platinoxide in methanol or sodium in methanol to give the tetrahydroquinoline product 2. The desired compound 3 may be prepared by reaction between 2 and e.g. an isothiocyanate (D-NCS) in the presence of a base such as lithium diisopropylamide in an appropriate solvent such as THF. Functional groups in 10 intermediates in reaction scheme I may be protected and deprotected using a strategy known in the art and described by e.g. T.W. Greene (Protective Groups in Organic Synthesis, 2nd Ed., John Wiley and Sons 1991). Compound 3 is a compound of formula I.

General Method B

Reaction Scheme II



Compounds of formula I may be prepared by the method shown in reaction scheme II starting with an amino acid ester of the type 4, which may either be commercially available or prepared by methods known to those skilled in the art e.g. R. Nagata et al. (J. Med. Chem. 1994, 37, 3956-3968) and a compound of the type D-NCS, e.g. isothiocyanate or isocyanate in the presence of a base such as lithium diisopropylamide in an appropriate solvent such as THF. The carboxylic acid 6 may be prepared from the ester 5 by methods known by those skilled in the art, e.g. with lithium hydroxide in an appropriate solvent such as dioxane/water. The desired compound 7 may be prepared from 6 and an amidated aminoacid by methods known by those skilled in the art, e.g. peptide coupling methodologies described in the art (e.g. DCC in DMF). Functional groups in intermediates in reaction scheme II may be protected and deprotected using a strategy known in the art and described by e.g. T.W. Greene (Protective Groups in Organic Synthesis, 2nd Ed., John Wiley and Sons 1991). Compound 7 is a compound of formula I.

Pharmaceutically acceptable acid addition salts of compounds of formula I include those prepared by reacting the compound with an inorganic or organic acid such as hydrochloric, hydrobromic, sulfuric, acetic, phosphoric, lactic, maleic, phthalic, citric, glutaric, gluconic, methanesulfonic, salicylic, succinic, tartaric, toluenesulfonic, trifluoroacetic, sulfamic or fumaric acid.

In another aspect, the present invention relates to a pharmaceutical composition comprising, as an active ingredient, a compound of the general formula I or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier or diluent.

Pharmaceutical compositions containing a compound of the present invention may be prepared by conventional techniques, e.g. as described in Remington's Pharmaceutical Sciences, 1985. The compositions may appear in conventional forms, for example 5 capsules, tablets, aerosols, solutions, suspensions or topical applications.

The pharmaceutical carrier or diluent employed may be a conventional solid or liquid carrier. Examples of solid carriers are lactose, terra alba, sucrose, cyclodextrin, talc, 10 gelatin, agar, pectin, acacia, magnesium stearate, stearic acid or lower alkyl ethers of cellulose. Examples of liquid carriers are syrup, peanut oil, olive oil, phospholipids, fatty acids, fatty acid amines, polyoxyethylene or water.

Similarly, the carrier or diluent may include any sustained 15 release material known in the art, such as glyceryl monostearate or glyceryl distearate, alone or mixed with a wax.

If a solid carrier is used for oral administration, the preparation may be tabletted, placed in a hard gelatin capsul in powder or pellet form or it can be in the form of a troche 20 or lozenge. The amount of solid carrier will vary widely but will usually be from about 25 mg to about 1 g. If a liquid carrier is used, the preparation may be in the form of a syrup, emulsion, soft gelatin capsule or sterile injectable liquid such as an aqueous or non-aqueous liquid suspension or 25 solution.

A typical tablet which may be prepared by conventional tableting techniques may contain:

Core:

Active compound (as free compound or salt thereof)	100mg
Colloidal silicon dioxide (Aerosil)	1.5mg
Cellulose, microcryst. (Avicel)	70mg
5 Modified cellulose gum (Ac-Di-Sol)	7.5mg
Magnesium stearate	

Coating:

HPMC approx.	9mg
10 *Mywacett 9-40 T approx.	0.9mg

*Acylated monoglyceride used as plasticizer for film coating.

For nasal administration, the preparation may contain a compound of formula I dissolved or suspended in a liquid carrier, in particular an aqueous carrier, for aerosol 15 application. The carrier may contain additives such as solubilizing agents, e.g. propylene glycol, surfactants, absorption enhancers such as lecithin (phosphatidylcholine) or cyclodextrin, or preservatives such as parabenes.

Generally, the compounds of the present invention are dispensed 20 in unit dosage form comprising 50-200 mg of active ingredient together with a pharmaceutically acceptable carrier per unit dosage.

The dosage of the compounds according to this invention is suitably 0.1-500 mg/day, e.g. from about 5 to about 50 mg, such 25 as about 10 mg per dose, when administered to patients, e.g. humans, as a drug.

It has been demonstrated that compounds of the general formula I possess the ability to release endogenous growth hormone in

vivo. The compounds may therefore be used in the treatment of conditions which require increased plasma growth hormone levels such as in growth hormone deficient humans or in elderly patients or livestock.

5 Thus, in a particular aspect, the present invention relates to a pharmaceutical composition for stimulating the release of growth hormone from the pituitary, the composition comprising, as an active ingredient, a compound of the general formula I or a pharmaceutically acceptable salt thereof together with a
10 pharmaceutically acceptable carrier or diluent.

In a further aspect, the present invention relates to a method of stimulating the release of growth hormone from the pituitary, the method comprising administering to a subject in need thereof an effective amount of a compound of the general
15 formula I or a pharmaceutically acceptable salt thereof.

In a still further aspect, the present invention relates to the use of a compound of the general formula I or a pharmaceutically acceptable salt thereof for the preparation of a medicament for stimulating the release of growth hormone from
20 the pituitary.

To those skilled in the art, it is well known that the current and potential uses of growth hormone in humans are varied and multitudinous. Thus, compounds of formula I can be administered for purposes stimulating release of growth hormone from the
25 pituitary and would then have similar effects or uses as growth hormone itself. The uses of growth hormone may be summarized as follows: stimulation of growth hormone release in the elderly; prevention of catabolic side effects of glucocorticoids, prevention and treatment of osteoporosis, stimulation of the
30 immune system, acceleration of wound healing, accelerating bone

fracture repair, treatment of growth retardation, treating renal failure or insufficiency resulting from growth retardation, treatment of physiological short stature including growth hormone deficient children and short stature associated with chronic illness, treatment of obesity and growth retardation associated with obesity, treating growth retardation associated with the Prader-Willi syndrome and Turner's syndrome; accelerating the recovery and reducing hospitalization of burn patients; treatment of intrauterine growth retardation, skeletal dysplasia, hypercortisolism and Cushing's syndrome; induction of pulsatile growth hormone release; replacement of growth hormone in stressed patients, treatment of osteochondrodysplasias, Noonan's syndrome, schizophrenia, depressions, Alzheimer's disease, delayed wound healing and psychosocial deprivation, treatment of pulmonary dysfunction and ventilator dependency, attenuation of protein catabolic responses after major surgery, reducing cachexia and protein loss due to chronic illness such as cancer or AIDS; treatment of hyperinsulinemia including nesidioblastosis, adjuvant treatment for ovulation induction; to stimulate thymic development and prevent the age-related decline of thymic function, treatment of immunosuppressed patients, improvement in muscle strength, mobility, maintenance of skin thickness, metabolic homeostasis, renal homeostasis in the frail elderly, stimulation of osteoblasts, bone remodelling and cartilage growth, stimulation of the immune system in companion animals and treatment of disorder of aging in companion animals, growth promoter in livestock and stimulation of wool growth in sheep.

For the above indications the dosage will vary depending on the compound of formula I employed, on the mode of administration and on the therapy desired. However, generally dosage levels between 0.0001 and 100 mg/kg body weight daily are administered to patients and animals to obtain effective release of

endogenous growth hormone. Usually, dosage forms suitable for oral, nasal, pulmonal or transdermal administration comprise from about 0.0001 mg to about 100 mg, preferably from about 0.001 mg to about 50 mg of the compounds of formula I admixed 5 with a pharmaceutically acceptable carrier or diluent.

The compounds of formula I may be administered in pharmaceutically acceptable acid addition salt form or, where appropriate, as a alkali metal or alkaline earth metal or lower alkylammonium salt. Such salt forms are believed to exhibit 10 approximately the same order of activity as the free base forms.

Optionally, the pharmaceutical composition of the invention may comprise a compound of formula I combined with one or more compounds exhibiting a different activity, e.g., an antibiotic 15 or other pharmacologically active material.

The route of administration may be any route which effectively transports the active compound to the appropriate or desired site of action, such as oral, nasal, pulmonary, transdermal or parenteral, the oral route being preferred.

20 Apart from the pharmaceutical use of the compounds of formula I, they may be useful in vitro tools for investigating the regulation of growth hormone release.

Compounds of formula I may also be useful in vivo tools for evaluating the growth hormone releasing capability of the 25 pituitary. For example, serum samples taken before and after administration of these compounds to humans can be assayed for growth hormone. Comparison of the growth hormone in each serum sample would directly determine the ability of the patients pituitary to release growth hormone.

Compounds of formula I may be administered to commercially important animals to increase their rate and extent of growth, and to increase milk production.

A further use of growth hormone secretagogue compounds of formula I is in combination with other secretagogues such as GHRP (2 or 6), GHRH and its analogues, growth hormone and its analogues or somatomedins including IGF-1 and IGF-2.

Pharmacological Methods

Compounds of formula I may be evaluated in vitro for their efficacy and potency to release growth hormone in rat pituitary primary cultures.

The isolation of rat pituitary cells is a modification of O. Sartor et al., Endocrinology 116, 1985, pp. 952-957. Male albino Sprague-Dawley rats (250 +/- 25 grams) were purchased from Møllegaard, Lille Skensved, Denmark. The rats were housed in group cages (four animals/cage) and placed in rooms with 12 hour light cycle. The room temperature varied from 19-24°C and the humidity from 30 - 60%.

The rats were decapitated and the pituitaries dissected. The neurointermediate lobes were removed and the remaining tissue was immediately placed in icecold isolation buffer (Gey's medium (Gibco 041-04030) supplemented with 0.25% D-glucose, 2% non-essential amino acids (Gibco 043-01140) and 1% bovine serum albumine (BSA) (Sigma A-4503)). The tissue was cut into small pieces and transferred to isolation buffer supplemented with 3.8 mg/ml of trypsin (Worthington #3707 TRL-3) and 330 mg/ml of DNase (Sigma D-4527). This mixture was incubated at 70 rotations/min for 35 min at 37°C in a 95/5% atmosphere of O₂/CO₂. The tissue was then washed three times in the above

buffer. Using a standard pasteur pipet, the tissue was then aspirated into single cells. After dispersion, cells were filtered through a nylon filter (160 mm) to remove undigested tissue. The cell suspension was washed 3 times with isolation buffer supplemented with trypsin inhibitor (0.75 mg/ml, Worthington #2829) and finally resuspended in culture medium; DMEM (Gibco 041-01965) supplemented with 25 mM HEPES (Sigma H-3375), 4 mM glutamine (Gibco 043-05030H), 0.075% sodium bicarbonate (Sigma S-8875), 0.1% non-essential amino acid, 2.5% fetal calf serum (FCS, Gibco 011-06290), 3% horse serum (Gibco 034-06050), 10% fresh rat serum, 1 nM T₃ (Sigma T-2752) and 40 mg/L dexamethasone (Sigma D-4902) pH 7.3, to a density of 2×10^5 cells/ml. The cells were seeded into microtiter plates (Nunc, Denmark), 200 ml/well, and cultured for 3 days at 37°C and 8% CO₂.

Compound testing

After culturing, the cells were washed twice with stimulation buffer (Hanks Balanced Salt Solution (Gibco 041-04020) supplemented with 1% BSA (Sigma A-4503), 0.25% D-glucose (Sigma G-5250) and 25 mM HEPES (Sigma H-3375) pH 7.3) and preincubated for 1 hour at 37°C. The buffer was exchanged with 90 ml stimulation buffer (37°C). Ten ml test compound solution was added and the plates were incubated for 15 min at 37°C and 5% CO₂. The medium was decanted and analyzed for GH content in an rGH SPA test system.

All compounds were tested in doses ranging from 10 pM to 100 mM. A dose-response relation was constructed using the Hill equation (Fig P, Biosoft). The efficacy (maximal GH released, E_{max}) was expressed in % of the E_{max} of GHRP-6. The potency (EC₅₀) was determined as the concentration inducing half maximal stimulation of the GH release.

Compounds of formula I may be evaluated for their metabolic stability.

Compounds were dissolved at a concentration of 1 mg/ml in water. 25 ml of this solution is added to 175 ml of the
5 respective enzyme-solution (resulting in an enzyme:substrate ratio (w/w) of approximately 1:5). The solution is left at 37°C overnight. 10 ml of the various degradation solutions is analyzed against a corresponding zero-sample using flow injection electrospray mass spectrometry (ESMS) with selected
10 ion monitoring of the molecular ion. If the signal has decreased more than 20% compared to the zero-sample, the remainder of the solution is analyzed by HPLC and mass spectrometry in order to identify the extent and site(s) of degradation precisely.

15 Several standard peptides (ACTH 4-10, Angiotensin 1-14 and Glucagon) have been included in the stability tests in order to verify the ability of the various solutions to degrade peptides.

Standard peptides (angiotensin 1-14, ACTH 4-10 and glucagon)
20 were purchased from Sigma, MO, USA)

Enzymes (trypsin, chymotrypsin, elastase aminopeptidase M and carboxypeptidase Y and B) were all purchased from Boehringer Mannheim GmbH (Mannheim, Germany)

Pancreatic enzyme mix: trypsin, chymotrypsin and elastase in
25 100 mM ammoniumbicarbonate pH 8.0 (all concentrations 0.025 mg/ml).

Carboxypeptidase mix: carboxypeptidase Y and B in 50 mM ammoniumacetate pH 4.5 (all concentrations 0.025 mg/ml).

Aminopeptidase M solution: aminopeptidase M (0.025 mg/ml) in 100 mM ammoniumbicarbonate pH 8.0

Mass spectrometric analysis was performed using two different mass spectrometers. A Sciex API III triple quadrupole LC-MS instrument (Sciex instruments, Thornhill, Ontario) equipped with an electrospray ion-source and a Bio-Ion 20 time-of-flight Plasma Desorption instrument (Bio-Ion Nordic AB, Uppsala, Sweden).

Quantification of the compounds (before and after degradation) was done on the API III instrument using single ion monitoring of the molecular ion in question with flow injection of the analyte. The liquid flow (MeOH:water 1:1) of 100 ml/min was controlled by an ABI 140B HPLC unit (Perkin-Elmer Applied Biosystems Divisions, Foster City, CA). The instrument parameters were set to standard operation conditions, and SIM monitoring was performed using the most intense molecular ion (in most cases this corresponded to the doubly charged molecular ion).

Identification of degradation products furthermore involved the use of plasma desorption mass spectrometry (PDMS) with sample application on nitrocellulose coated targets and standard instrumental settings. The accuracy of the hereby determined masses is generally better than 0.1%.

Separation and isolation of degradation products was done using a HY-TACH C-18 reverse phase 4.6x105 mm HPLC column (Hewlett-Packard Company, Palo Alto, CA) with a standard acetonitril: TFA separation gradient. The HPLC system used was HP1090M (Hewlett-Packard Company, Palo Alto, CA).

Peptide derivative	MW/ SIM ion (amu)	Ca r b o x y - p e p t i d a s e m i x	P a n . enzyme mix
Standards			
ACTH 4-10	1124. 5/562 .8	+	-
Glucagon	3483. 871.8	-	-
Insulin (B23-29)	859.1 /430. 6		
Angiotensin 1-14	1760. 1/881 .0	-	-
GHRP-2	817.4 /409. 6	-	-
GHRP-6	872.6 /437. 4	-	-

+: Stable (less than 20% decrease in SIM signal after 24 h in degradation solution)

-: Unstable (more than 20% decrease in SIM signal after 24 h in degradation solution)

Any novel feature or combination of features described herein is considered essential to this invention.

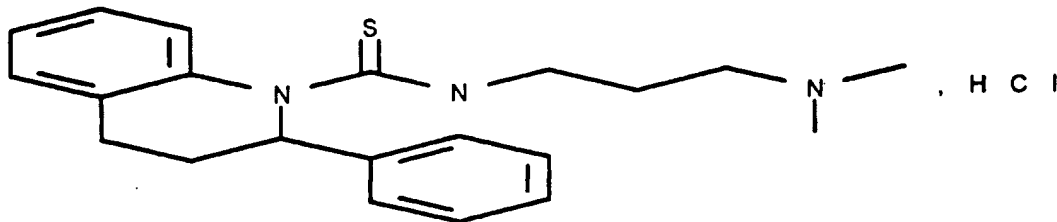
The invention is further illustrated in the following examples which are not in any way intended to limit the scope of the invention as claimed.

EXAMPLES

Hereinafter, TLC is thin layer chromatography and THF is tetrahydrofuran, CDCl_3 is deuterio chloroform, $\text{DMSO}-d_6$ is hexadeuterio dimethylsulfoxide and CD_3OD is tetradeuterio methanol. The structure of the compounds are confirmed by either elemental analysis or NMR, where peaks assigned to characteristic protons in the title compounds are presented where appropriate. ^1H NMR shift (δ) are given in parts per million (ppm). M.p. is melting point and is given in $^\circ\text{C}$ and is not corrected. Column chromatography was carried out using the technique described by W.C. Still et al., J. Org. Chem. (1978), 43, 2923-2925 on Merck silica gel 60 (Art. 9385). HPLC analysis was performed using a 5mm C18 4x250 mm column, eluting with 20-80 % gradient of 0.1 % trifluoroacetic acid/acetonitrile and 0.1 % trifluoroacetic acid/water over 30 minutes at 35 $^\circ\text{C}$. All reactions were carried out under an atmosphere of nitrogen. THF was distilled over sodium and benzophenone before use. Compounds used as starting material are either known compounds or compounds which can readily be prepared by methods known per se.

Example 1

2-Phenyl-3,4-dihydro-2H-quinoline-1-carbothioic acid (3-(dimethylamino)propyl)amide -hydrochloride



To a solution of 2-phenylquinoline (10.0 g, 50 mmol) in 250 ml 1-propanol under reflux was added sodium (11.5 g, 0.5 mol) in portions over a period of 30 minutes. After 2 hours at reflux the solution was cooled and 50 ml of water was slowly added. 5 The mixture was concentrated in vacuo to an oil which was dissolved in 300 ml of methylene chloride and washed 4 times with 200 ml of water. The organic layer was dried over magnesium sulphate, filtered and concentrated in vacuo to give 8.2 g (78 %) of 2-phenyl-1,2,3,4-tetrahydrohydro-2H-quinoline.

10 To a solution of 2-phenyl-1,2,3,4-tetrahydrohydro-2H-quinoline (0.5 g, 2.4 mmol) in 15 ml of THF at -78°C was added lithium diisopropylamide (1.3 ml of a 2.0 M solution in THF). After 10 minutes 3-(dimethylamino)propyl isothiocyanate (0.2, 2.6 mmol) in 10 ml of THF was added and the mixture was stirred 15 overnight. The mixture was concentrated in vacuo to a oil which was chromatographed on 500 ml of silicagel with 20 % methanol/methylene chloride to give a product which was dissolved in 20 ml of ethyl acetate to which was added 10 ml of 3M HCl in ethyl acetate. The mixture was concentrated in vacuo 20 to give 300 mg (32 %) of 2-phenyl-1,2,3,4-tetrahydrohydro-2H-quinoline-1-carbothioic acid (3-(dimethylamino)propyl)amide-hydrochloride as an amorphous powder.

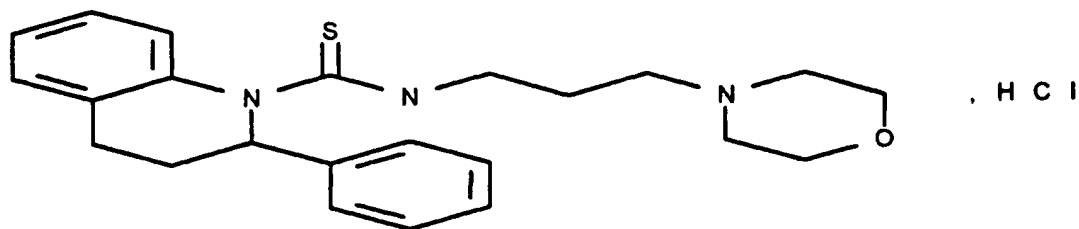
¹H NMR (400 MHz, CD₃OD): d 2.00 (m, 2H), 2.15 (m, 1H), 2.55 (m, 1H), 2.70 (m, 2H), 2.85 (s, 3H), 2.90 (s, 3H), 3.15 (t, 2H), 25 3.75 (m, 2H), 6.55 (t, 1H), 7.1-7.4 (m, 9H).

Calculated for C₂₁H₂₇N₃S.HCl, 3/2 H₂O: 60.4 %; H, 7.4 %; N, 10.1 %
Found: C, 60.3 %; H, 7.4 %; N, 10.0 %

Reverse Phase HPLC: 21 min.

Example 2

2-Phenyl-3,4-dihydro-2H-quinoline-1-carbothioic acid (3-(morpholin-4-yl)propyl) amide-hydrochloride



5 To a solution of 2-phenyl-1,2,3,4-tetrahydrohydro-2H-quinoline (0.7 g, 3.3 mmol, prepared as in example 1), in 30 ml of THF at -78°C was added lithium diisopropylamide (1.8 ml of a 2.0 M solution in THF). After 15 minutes 3-(morpholin-4-yl)propyl isothiocyanate (0.6 g, 3.3 mmol) in 20 ml of THF was added and
10 the mixture was stirred overnight. The mixture was concentrated in vacuo to an oil which was chromatographed on 500 ml of silicagel with 5 % methanol/methylene chloride to give a product which was dissolved in 20 ml of ethyl acetate to which was added 10 ml of 3M HCl in ethyl acetate. The mixture was
15 concentrated in vacuo to give 416 mg (29 %) of 2-phenyl-1,2,3,4-tetrahydrohydro-2H-quinoline-1-carbothioic acid (3-(morpholin-4-yl)propyl)amide-hydrochloride as an amorphous powder.

¹H NMR (400 MHz, CDCl₃, free base): δ 1.70 (m, 2H), 2.15 (m, 20 1H), 2.25 (t, 4H), 2.35 (m, 1H), 2.55 (m, 1H), 2.65 (t, 2H), 3.30 (t, 4H), 3.65 (m, 2H), 3.95 (m, 1H), 6.60 (t, 1H), 7.1-7.4 (m, 9H)

Calculated for $C_{23}H_{29}N_3OS \cdot HCl, 3/2 H_2O$:

C, 60.1 %; H, 7.2 %; N, 9.2 %

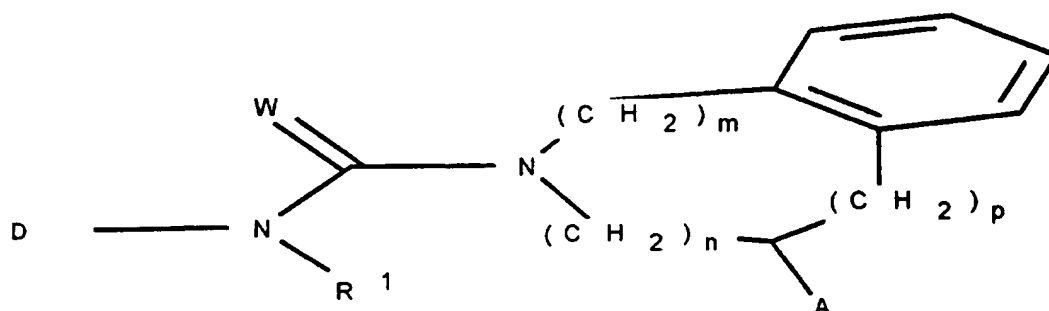
Found:

C, 60.0 %; H, 7.6 %; N, 9.5 %

Reverse Phase HPLC: 21 min.

CLAIMS

1. A compound of general formula I



I

5 wherein

m, n and p are independently 0, 1 or 2,

m + n + p is 2,

W is =S, =O, =NH or =N(CN),

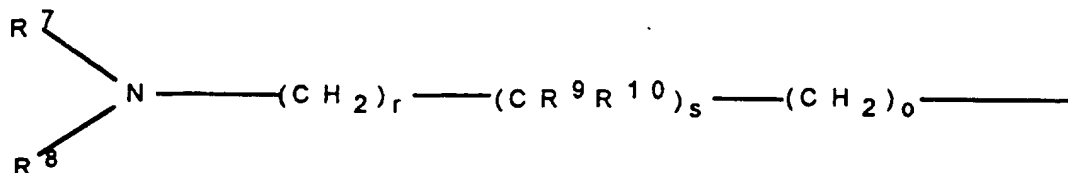
R¹ is hydrogen, aryl or C₁₋₆-alkyl optionally substituted with
10 halogen, amino, hydroxy or aryl;

A is aryl or C₁₋₆-alkyl optionally substituted with aryl, -
CONR³R⁴ or

-CONR³CHR⁴CONR⁵R⁶, wherein R³, R⁴, R⁵, and R⁶ independently are
15 hydrogen, aryl or C₁₋₆-alkyl optionally substituted with

halogen, amino, hydroxy or aryl,
with the proviso that at least one of R³, R⁴, R⁵ or R⁶ is an aryl
or C₁₋₆-alkyl substituted with aryl;

D is



wherein R^7 , R^8 , R^9 and R^{10} independently are hydrogen or C_{1-6} -alkyl optionally substituted with halogen, amino, hydroxy or aryl, R^7 and R^8 , R^9 and R^{10} , R^9 and R^{10} optionally forming $-(\text{CH}_2)_i-\text{U}-(\text{CH}_2)_j-$, wherein i and j are independently 1, 2 or 3,

U is -O-, -S- or a valence bond,

o and r are independently 0, 1, 2, 3 or 4,

s is 0 or 1, and

$r + s + o$ is 2, 3 or 4;

or a pharmaceutically acceptable salt thereof, and the compounds of formula I comprise any optical isomers thereof, in the form of separated, pure or partially purified optical isomers or racemic mixtures thereof.

2. A compound of the general formula I according to claim 1, wherein W is =S or =O;

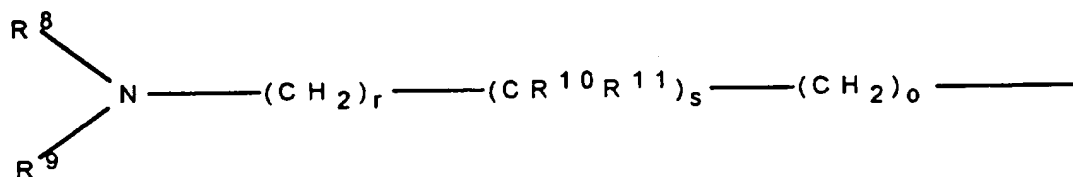
and A, D, R^1 , m, n and p are defined as in the preceding claims.

3. A compound of the general formula I according to any one of the preceding claims, wherein A is aryl or C_{1-6} -alkyl optionally substituted with aryl;

and D, R^1 , W, m, n and p are defined as in the preceding claims.

4. A compound of the general formula I according to any one of the preceding claims, wherein A is CONR^3R^4 and R^3 and R^4 independently are hydrogen, aryl or C_{1-6} -alkyl optionally substituted with halogen, amino, hydroxy or aryl;
- 5 and D, R^1 , W, m, n and p are defined as in the preceding claims.

5. A compound of the general formula I according to the preceding claims, wherein D is



- 10 wherein R^{10} and R^{11} are hydrogen;
 R^8 and R^9 are independently hydrogen or C_{1-6} -alkyl optionally substituted with halogen, amino, hydroxy or aryl;
 R^6 and R^9 , optionally forming $-(\text{CH}_2)_i\text{-U-(CH}_2)_j\text{-}$, wherein i and j are independently 1 or 2,
- 15 U is -O-, -S- or a valence bond,
 o and r are independently 0, 1, 2, 3 or 4,
 s is 0 or 1, and
 $r + s + o$ is 2, 3 or 4;
 and A, R^1 , W, m, n and p are defined as in the preceding
- 20 claims.

6. A compound according to the claims 1, 2, 3, 4 or 5 selected from the group consisting of

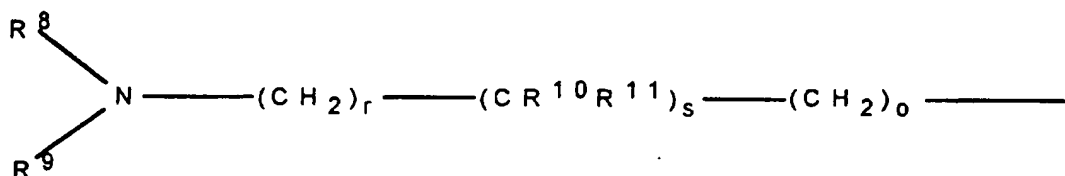
2-Phenyl-3,4-dihydro-2H-quinoline-1-carbothioic acid (3-(dimethylamino) propyl) amide, or the hydrochloride salt

25 thereof;

Benzyl-3,4-dihydro-2H-quinoline-1-carbothioic acid (3-(dimethylamino)propyl) amide;
 1-(3-((Morpholin-4-yl)propyl)thiocarbamoyl)-1,2,3,4-tetrahydro-1H-quinoline-2-carboxylic acid N-(1-carbamoyl-2-(naphth-1-yl)ethyl)-N-methylamide;
 2-(3-((Morpholin-4-yl)propyl)thiocarbamoyl)-1,2,3,4-tetrahydro-1H-isoquinoline-3-carboxylic acid N-[1-((4-aminobutyl)carbamoyl)-2-(naphth-1-yl)ethyl]-N-methylamide; or
 2-Phenyl-3,4-dihydro-2H-quinoline-1-carbothioic acid (3-(morpholin-4-yl)propyl) amide, or the hydrochloride salt thereof.

7. The compound of the general formula 3 wherein D and A are as defined in claim 1; or a pharmaceutically acceptable salt thereof, and the compounds of formula I comprise any optical isomers thereof, in the form of separated, pure or partially purified optical isomers or racemic mixtures thereof.

8. The compound according to claim 7, wherein D is



wherein R^{10} and R^{11} are hydrogen;

R^8 and R^9 are independently hydrogen or C_{1-6} -alkyl optionally substituted with halogen, amino, hydroxy or aryl;

R^8 and R^9 , optionally forming $-(\text{CH}_2)_i\text{-U-(CH}_2)_j\text{-}$, wherein i and j are independently 1 or 2,

U is -O-, -S- or a valence bond,

o and r are independently 0, 1, 2, 3 or 4,

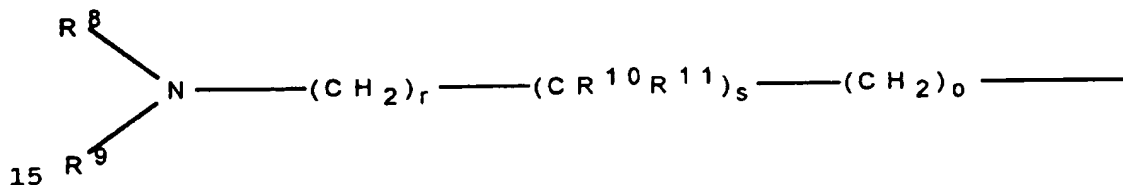
s is 0 or 1, and
r + s + o is 2, 3 or 4.

9. The compound according to any one of the claims 7 or 8, wherein A is CONR^3R^4 and R^3 and R^4 independently are hydrogen, 5 aryl or C_{1-6} -alkyl optionally substituted with halogen, amino, hydroxy or aryl.

10. A compound of the general formula 7 wherein D, R^3 , R^4 , R^5 , R^6 , m, n, and p are as defined in claim 1;

10 or a pharmaceutically acceptable salt thereof, and the compounds of formula I comprise any optical isomers thereof, in the form of separated, pure or partially purified optical isomers or racemic mixtures thereof.

11. The compound according to claim 10, wherein D is



wherein R^{10} and R^{11} are hydrogen;

R^8 and R^9 are independently hydrogen or C_{1-6} -alkyl optionally substituted with halogen, amino, hydroxy or aryl;

R^8 and R^9 , optionally forming $-(\text{CH}_2)_i\text{---U---}(\text{CH}_2)_j\text{---}$, wherein i and j 20 are independently 1 or 2,

U is $-\text{O}-$, $-\text{S}-$ or a valence bond,

o and r are independently 0, 1, 2, 3 or 4,

s is 0 or 1, and

r + s + o is 2, 3 or 4.

12. A pharmaceutical composition comprising, as an active ingredient, a compound of the general formula I according to any one of the claims 1-11 or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier or diluent.

13. The composition according to claim 12 in unit dosage form, comprising from about 10 to about 200 mg of the compound of the general formula I or a pharmaceutically acceptable salt thereof.

10 14. A pharmaceutical composition for stimulating the release of growth hormone from the pituitary, the composition comprising, as an active ingredient, a compound of the general formula I according to any one of the claims 1-11 or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier or diluent.

15. A method of stimulating the release of growth hormone from the pituitary, the method comprising administering to a subject in need thereof an effective amount of a compound of the general formula I according to any one of the claims 1-11 or a pharmaceutically acceptable salt thereof.

16. The method according to claim 15, wherein the effective amount of the compound of the general formula I or pharmaceutically acceptable salt or ester thereof is in the range of from about 0.0001 to about 100 mg/kg body weight per day, preferably from about 0.001 to about 50 mg/kg body weight per day.

17. Use of a compound of the general formula I according to any one of the claims 1-11 or a pharmaceutically acceptable salt thereof for the preparation of a medicament.

18. Use of a compound of the general formula I according to any one of the claims 1-11 or a pharmaceutically acceptable salt thereof for the preparation of a medicament for stimulating the release of growth hormone from the pituitary.

5 19. Use of a compound of the general formula I according to any one of the claims 1-11 or a pharmaceutically acceptable salt thereof for the preparation of a medicament for administration to animals to increase their rate and extent of growth, to increase their milk and wool production, or for the treatment
10 of ailments.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 96/00059

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07D 215/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

FILE REG, CA, CA PLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

F document member of the same patent family

Date of the actual completion of the international search

10 June 1996

Date of mailing of the international search report

11.06.95

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK96/00059

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 15-16
because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv).: Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.